4-(HETEROCYCLYL - FUSED PHENYL)-3-(PHENYL OR PYRID -2-YL)PYRAZOLES AS INHIBITORS OF THE ALK -5 RECEPTOR

Field of the Invention

This invention relates to novel pyrazole derivatives which are inhibitors of the transforming growth factor, ("TGF")-ß signalling pathway, in particular, the phosphorylation of smad2 or smad3 by the TGF-β type I or activin-like kinase ("ALK")-5 receptor, methods for their preparation and their use in medicine, specifically in the treatment and prevention of a disease state mediated by this pathway.

Background of the Invention

TGF-β1 is the prototypic member of a family of cytokines including the TGF-βs, activins, inhibins, bone morphogenetic proteins and Müllerian-inhibiting substance, that signal through a family of single transmembrane serine/threonine kinase receptors. These receptors can be divided into two classes, the type I or activin like kinase (ALK) receptors and type II receptors. The ALK receptors are distinguished from the type II receptors in that the ALK receptors (a) lack the serine/threonine rich intracellular tail, (b) possess serine/threonine kinase domains that are very homologous between type I receptors, and (c) share a common sequence motif called the GS domain, consisting of a region rich in glycine and serine residues. The GS domain is at the amino terminal end of the intracellular kinase domain and is critical for activation by the type II receptor. Several studies have shown that TGF-β signaling requires both the ALK and type II receptors. Specifically, the type II receptor phosphorylates the GS domain of the type I receptor for TGF-β, ALK5, in the presence of TGF-β. The ALK5, in turn, phosphorylates the cytoplasmic proteins smad2 and smad3 at two carboxy terminal serines. The phosphorylated smad proteins translocate into the nucleus and activate genes that contribute to the production of extracellular matrix. Therefore, preferred compounds of this invention are selective in that they inhibit the type I receptor and thus matrix production.

WO02/066462, WO02/062794 and WO02/062787 (Glaxo Group Limited) disclose novel substituted pyrazole derivatives which are inhibitors of the transforming growth factor, ("TGF")-ß signaling pathway, in particular, the phosphorylation of smad2 or

smad3 by the TGF-β type I or activin-like kinase ("ALK")-5 receptor. The compounds are said to have utility in the treatment and prevention of various disease states mediated by ALK5 kinase mechanisms.

WO04/016606 (SmithKline Beecham Corporation) discloses phenyl pyridyl substituted pyrazole derivatives which are inhibitors of the transforming growth factor, ("TGF")-ß signaling pathway, in particular, the phosphorylation of smad2 or smad3 by the TGF-β type I or activin-like kinase ("ALK")-5 receptor.

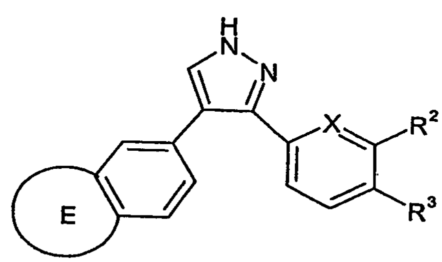
WO2004/026306 (Pfizer) describes novel pyrazole compounds which are potent inhibitors of transforming growth factor ("TGF")-ß signalling pathway. The compounds are said to be useful in the treatment of various TGF-related disease states including, for example, cancer and fibrotic diseases.

Summary of the Invention

Surprisingly, it has now been discovered that a class of novel pyrazoles derivatives function as potent and selective non-peptide inhibitors of ALK5 kinase.

Detailed Description of the Invention

According to a first aspect, the invention provides the use of a compound of formula (I), a pharmaceutically acceptable salt, solvate or derivative thereof;



(I)

wherein

ring E is a saturated, unsaturated or aromatic 5 or 6-membered heterocycle which heterocycle in addition to carbon contains one or more ring-heteroatoms independently selected from nitrogen and oxygen, wherein the heterocycle is optionally substituted on any nitrogen atom where appropriate by one or more groups R^{Ea} independently selected from C_{1-6}alkyl and C_{1-6}alkoxyC_{1-6}alkyl and is optionally

substituted on any carbon atom where appropriate by one or more groups R^{Eb} independently selected from oxo, C_{1-6}alkyl, C_{1-6}alkoxyC_{1-6}alkyl, C_{1-6}alkoxy and halo;
X is N or CH;
R^2 is hydrogen, C_{1-6}alkyl, halo, cyano or perfluoroC_{1-6}alkyl; and
R^3 is hydrogen or halo;
in the preparation of a medicament for treating or preventing a disease or condition mediated by ALK-5 inhibition.

Preferably the benzofused ring system including E is selected from the list: benzimidazol-6-yl, benzimidazol-5-yl, benzoxazol-6-yl, benzoxazol-5-yl, 4H-benzo[1,4]oxazin-3-one-6-yl, benzo[1,3]dioxol-5-yl, benzodioxan-6-yl, quinolin-6-yl and benzotriazol-6-yl.

More preferably the benzofused ring system including E is selected from the list: benzimidazol-6-yl, benzimidazol-5-yl, benzoxazol-6-yl, benzoxazol-5-yl, 4H-benzo[1,4]oxazin-3-one-6-yl and benzodioxan-6-yl.

Preferably X is N or CH. More preferably, X is N.

Preferably R^2 is hydrogen, C_{1-6}alkyl, chloro or fluoro. More preferably R^2 is hydrogen, methyl, chloro or fluoro. More preferably still, R^2 is methyl.

Preferably R^3 is hydrogen.

Preferably, when X is N, R^2 is methyl. More preferably when X is N and R^2 is methyl, R^3 is H.

Preferably, when X is CH, R^2 is chloro. More preferably, when X is CH and R^2 is chloro, R^3 is H.

It will be appreciated that the present invention is intended to include compounds having any combination of the preferred groups listed hereinbefore.

The term "ALK5 inhibitor" is used herein to mean a compound, other than inhibitory smads, e.g. smad6 and smad7, which selectively inhibits the ALK5 receptor preferentially over p38 or type II receptors.

Activation of the TGF-β1 axis and expansion of extracellular matrix are early and persistent contributors to the development and progression of chronic renal disease and vascular disease. Border W.A., et al, N. Engl. J. Med., 1994; 331(19), 1286-92. Further, TGF-β1 plays a role in the formation of fibronectin and plasminogen activator inhibitor-1, components of sclerotic deposits, through the action of smad3 phosphorylation by the TGF-β1 receptor ALK5. Zhang Y., et al, Nature, 1998; 394(6696), 909-13; Usui T., et al, Invest. Ophthalmol. Vis. Sci., 1998; 39(11), 1981-9.

Progressive fibrosis in the kidney and cardiovascular system is a major cause of suffering and death and an important contributor to the cost of health care. TGF-β1 has been implicated in many renal fibrotic disorders. Border W.A., et al, N. Engl. J. Med., 1994; 331(19), 1286-92. TGF-β1 is elevated in acute and chronic glomerulonephritis Yoshioka K., et al, Lab. Invest., 1993; 68(2), 154-63, diabetic nephropathy Yamamoto, T., et al, 1993, PNAS 90, 1814-1818., allograft rejection, HIV nephropathy and angiotensin-induced nephropathy Border W.A., et al, N. Engl. J. Med., 1994; 331(19), 1286-92. In these diseases the levels of TGF-β1 expression coincide with the production of extracellular matrix. Three lines of evidence suggest a causal relationship between TGF-β1 and the production of matrix. First, normal glomeruli, mesangial cells and non-renal cells can be induced to produce extracellular-matrix protein and inhibit protease activity by exogenous TGF-β1 in vitro. Second, neutralising anti-bodies against TGF-β1 can prevent the accumulation of extracellular matrix in nephritic rats. Third, TGF-β1 transgenic mice or in vivo transfection of the TGF-β1 gene into normal rat kidneys resulted in the rapid development of glomerulosclerosis. Kopp J.B., et al, Lab. Invest., 1996; 74(6), 991-1003. Thus, inhibition of TGF-β1 activity is indicated as a therapeutic intervention in chronic renal disease.

TGF-β1 and its receptors are increased in injured blood vessels and are indicated in neointima formation following balloon angioplasty Saltis J., et al, Clin. Exp. Pharmacol. Physiol., 1996; 23(3), 193-200. In addition TGF-β1 is a potent stimulator of smooth muscle cell ("SMC") migration in vitro and migration of SMC in the arterial wall is a contributing factor in the pathogenesis of atherosclerosis and restenosis. Moreover, in multivariate analysis of the endothelial cell products against total cholesterol, TGF-β receptor ALK5 correlated with total cholesterol ($P < 0.001$) Blann

A.D., et al, Atherosclerosis, 1996; 120(1-2), 221-6. Furthermore, SMC derived from human atherosclerotic lesions have an increased ALK5/TGF-β type II receptor ratio. Because TGF-β1 is over-expressed in fibroproliferative vascular lesions, receptor-variant cells would be allowed to grow in a slow, but uncontrolled fashion, while overproducing extracellular matrix components McCaffrey T.A., et al, Jr., J. Clin. Invest., 1995; 96(6), 2667-75. TGF-β1 was immunolocalized to non-foamy macrophages in atherosclerotic lesions where active matrix synthesis occurs, suggesting that non-foamy macrophages may participate in modulating matrix gene expression in atherosclerotic remodeling via a TGF-β-dependent mechanism. Therefore, inhibiting the action of TGF-β1 on ALK5 is also indicated in atherosclerosis and restenosis.

TGF-β is also indicated in wound repair. Neutralising antibodies to TGF-β1 have been used in a number of models to illustrate that inhibition of TGF-β1 signalling is beneficial in restoring function after injury by limiting excessive scar formation during the healing process. For example, neutralising antibodies to TGF-β1 and TGF-β2 reduced scar formation and improved the cytoarchitecture of the neodermis by reducing the number of monocytes and macrophages as well as decreasing dermal fibronectin and collagen deposition in rats Shah M., J. Cell. Sci., 1995, 108, 985-1002. Moreover, TGF-β antibodies also improve healing of corneal wounds in rabbits Moller-Pedersen T., Curr. Eye Res., 1998, 17, 736-747, and accelerate wound healing of gastric ulcers in the rat, Ernst H., Gut, 1996, 39, 172-175. These data strongly suggest that limiting the activity of TGF-β would be beneficial in many tissues and suggest that any disease with chronic elevation of TGF-β would benefit by inhibiting smad2 and smad3 signaling pathways.

TGF-β is also implicated in photoaging of the skin (see Fisher GJ. Kang SW. Varani J. Bata-Csorgo Z. Wan YS. Data S. Voorhees JJ. , Mechanisms of photoaging and chronological skin aging, *Archives of Dermatology*, 138(11):1462-1470, 2002 Nov. and Schwartz E. Sapadin AN. Kligman LH. "Ultraviolet B radiation increases steady state mRNA levels for cytokines and integrins in hairless mouse skin- modulation by topical tretinoin", Archives if Dermatological Research, 290(3):137-144, 1998 Mar.)

TGF-β is also implicated in peritoneal adhesions Saed G.M., et al, Wound Repair Regeneration, 1999 Nov-Dec, 7(6), 504-510. Therefore, inhibitors of ALK5 would be

beneficial in preventing peritoneal and sub-dermal fibrotic adhesions following surgical procedures.

Therefore a disease or condition mediated by ALK-5 inhibition is preferably selected from the list: chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers (including diabetic ulcers, chronic ulcers, gastric ulcers, and duodenal ulcers), ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, atherosclerosis, peritoneal , sub-dermal adhesion and photoaging, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, for example, hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol-induced hepatitis, haemochromatosis and primary biliary cirrhosis, restenosis, retroperitoneal fibrosis, mesenteric fibrosis, endometriosis and keloids.

More preferably the disease or condition mediated by ALK-5 inhibition is fibrosis. Preferably kidney fibrosis.

Many of the compounds of formula (I) are novel. Therefore according to a second aspect the invention provides a compound as defined in the first aspect with the proviso that compound of formula (I) is not:

1,3-diethyl-1,3-dihydro-5-[3-(3-methylphenyl)-1H-pyrazol-4-yl]-2H-benzimidazol-2-one;

1,3-dihydro-1,3-dimethyl-5-(3-phenyl-1H-pyrazol-4-yl)-2H-benzimidazol-2-one;

1,3-diethyl-1,3-dihydro-5-(3-phenyl-1H-pyrazol-4-yl)-2H-benzimidazol-2-one;

1-ethyl-1,3-dihydro-3-methyl-5-[3-(3-methylphenyl)-1H-pyrazol-4-yl]-2H-enzimidazol-2-one;

1,3-diethyl-5-[3-(4-fluoro-3-methylphenyl)-1H-pyrazol-4-yl]-1,3-dihydro-2H-benzimidazol-2-one;

1,3-diethyl-5-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-1,3-dihydro-2H-benzimidazol-2-one;

1,3-diethyl-1,3-dihydro-5-[3-[3-(trifluoromethyl)phenyl]-1H-pyrazol-4-yl]-2H-benzimidazol-2-one;

1-ethyl-6-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-1H-benzimidazole;

1-ethyl-5-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-1H-benzimidazole;

6-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-1-(1-methylethyl)-1H-benzimidazole;

5-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-1-(1-methylethyl)-1H-benzimidazole;

6-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-1-(2-propoxyethyl)-1H-benzimidazole;

6-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-1-(1-propoxyethyl)-1H-benzimidazole;
6-[3-(4-bromophenyl)-1H-pyrazol-4-yl]-1-methyl-1H-benzimidazole;
6-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-1-methyl-1H-benzimidazole;
1-methyl-6-[3-(2-pyridinyl)-1H-pyrazol-4-yl]-1H-benzimidazole;
6-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-1-methyl-1H-benzimidazole; or
5-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-1-methyl-1H-benzimidazole.

According to a third aspect, the invention provides a compound as defined in the first aspect with the provisos that a) when the benzofused ring system including E is 1,3-dihydro-2H-benzimidazol-2-one-5-yl, X is not CH; b) when the benzofused ring system including E is benzimidazol-5-yl or benzimidazol-6-yl and X is CH, R^3 is not halogen, and c) when the benzofused ring system including E is benzimidazol-6-yl and X is N, R^2 is not hydrogen.

According to a fourth aspect, the invention provides a compound as defined in the first aspect with the proviso that a) when the benzofused ring system including E is 1,3-dihydro-2H-benzimidazol-2-one-5-yl, X is not CH; and b) when the benzofused ring system including E is benzimidazol-5-yl or benzimidazol-6-yl, R^2 is not hydrogen.

Compounds of formula (I) which are of special interest as agents useful in the treatment or prophylaxis of disorders characterised by the overexpression of TGF-β are selected from the list:

4-[1-ethyl-benzimidazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole (Example 1);
4-[1-(2-methoxyethyl)-benzimidazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole (Example 2);
4-[1-(methyl)-benzimidazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole (Example 3);
4-[2-(methyl)-benzoxazol-6-yl]-3-[pyridin-2-yl]-1H-pyrazole (Example 4);
4-[2-(methyl)-benzoxazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole (Example 5);
4-[benzoxazol-6-yl]-3-[pyridin-2-yl]-1H-pyrazole (Example 6);
4-[benzoxazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole (Example 7);
4-[benzoxazol-5-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole (Example 8);
4-[2-methyl-benzoxazol-5-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole (Example 9);
4-[4-methyl-4H-benzo[1,4]oxazin-3-one-6-yl]-3-(6-methylpyridin-2-yl)-1H-pyrazole (Example 10);

4-[4-ethyl-4H-benzo[1,4]oxazin-3-one-6-yl]-3-(6-methylpyridin-2-yl)-1H-pyrazole (Example 11);

4-[4H-benzo[1,4]oxazin-3-one-6-yl]-3-(3-chlorophenyl)-1H-pyrazole (Example 12);

4-[4-ethyl-4H-benzo[1,4]oxazin-3-one-6-yl]-3-(3-chlorophenyl)-1H-pyrazole (Example 13);

and pharmaceutically acceptable salts, solvates and derivatives thereof.

The term "C_{1-6}alkyl" as used herein, whether on its own or as part of a group, refers to a straight or branched chain saturated aliphatic hydrocarbon radical of 1 to 6 carbon atoms, unless the chain length is limited thereto, including, but not limited to methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, pentyl and hexyl.

The term "alkoxy" as a group or part of a group refers to an alkyl ether radical, wherein the term "alkyl" is defined above. Such alkoxy groups in particular include methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, iso-butoxy, sec-butoxy and tert-butoxy.

The term "perfluoroalkyl" as used herein includes compounds such as trifluoromethyl.

The terms "halo" or "halogen" are used interchangeably herein to mean radicals derived from the elements chlorine, fluorine, iodine and bromine.

For the avoidance of doubt, unless otherwise indicated, the term substituted means substituted by one or more defined groups. In the case where groups may be selected from a number of alternative groups, the selected groups may be the same or different.

For the avoidance of doubt, the term independently means that where more than one substituent is selected from a number of possible substituents, those substituents may be the same or different.

As used herein the term "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, solvate, ester or amide, or salt or solvate of such ester or amide, of the compound of formula (I), or any other compound which upon administration to the recipient is capable of providing (directly or indirectly) a

compound of formula (I) or an active metabolite or residue thereof, eg, a prodrug. Preferred pharmaceutically acceptable derivatives according to the invention are any pharmaceutically acceptable salts, solvates or prodrugs.

Suitable pharmaceutically acceptable salts of the compounds of formula (I) include acid salts, for example sodium, potassium, calcium, magnesium and tetraalkylammonium and the like, or mono- or di- basic salts with the appropriate acid for example organic carboxylic acids such as acetic, lactic, tartaric, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids and inorganic acids such as hydrochloric, sulfuric, phosphoric and sulfamic acids and the like. Some of the compounds of this invention may be crystallised or recrystallised from solvents such as aqueous and organic solvents. In such cases solvates may be formed. This invention includes within its scope stoichiometric solvates including hydrates as well as compounds containing variable amounts of water that may be produced by processes such as lyophilisation.

Hereinafter, compounds, their pharmaceutically acceptable salts, their solvates and polymorphs, defined in any aspect of the invention (except intermediate compounds in chemical processes) are referred to as "compounds of the invention".

Compounds of the invention may exist in the form of optical isomers, e.g. diastereoisomers and mixtures of isomers in all ratios, e.g. racemic mixtures. The invention includes all such forms, in particular the pure isomeric forms. The different isomeric forms may be separated or resolved one from the other by conventional methods, or any given isomer may be obtained by conventional synthetic methods or by stereospecific or asymmetric syntheses.

The compounds of the invention may exist in one or more tautomeric forms. All tautomers and mixtures therof are included in the scope of the present invention.

Since the compounds of the invention are intended for use in pharmaceutical compositions it will readily be understood that they are each preferably provided in substantially pure form, for example at least 60% pure, more suitably at least 75% pure and preferably at least 85%, especially at least 98% pure (% are on a weight for weight basis). Impure preparations of the compounds may be used for preparing the

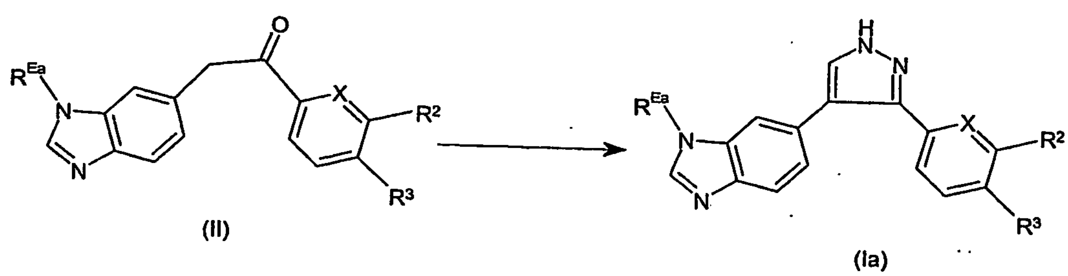
more pure forms used in the pharmaceutical compositions; these less pure preparations of the compounds should contain at least 1%, more suitably at least 5% and preferably from 10 to 59% of a compound of the invention.

Compounds of the invention may be prepared, in known manner in a variety of ways. In the following reaction schemes and hereafter, unless otherwise stated R^1 to R^3, X and E are as defined in the first aspect. These processes form further aspects of the invention.

Throughout the specification, general formulae are designated by Roman numerals (I), (II), (III), (IV) etc. Subsets of these general formulae are defined as (Ia), (Ib), (Ic) etc .... (IVa), (IVb), (IVc) etc.

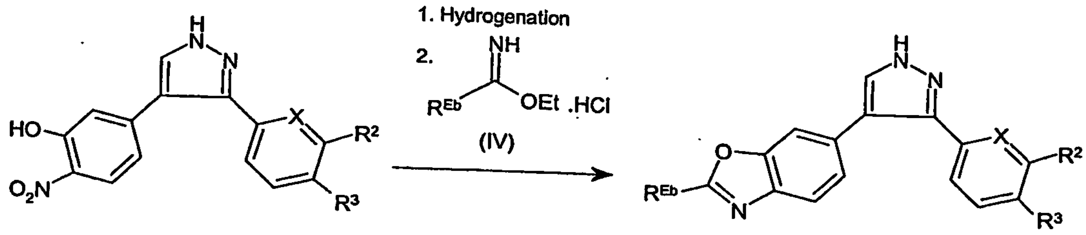
Compounds of formula (Ia), i.e. compounds of general formula (I) where the benzofused ring system including E is benzimidazol-6-yl, may be prepared from compounds of formula (II) according to reaction scheme 1 by treating (II) with N,N-dimethylformamide dimethyl acetal in THF and acetic acid at room temperature followed by addition of hydrazine at room temperature.

Scheme 1



Compounds of formula (Ib), i.e. compounds of general formula (I) where the benzofused ring system including E is benzoxazol-6-yl, may be prepared from compounds of formula (III) according to reaction scheme 2 by treating (III) with hydrogen in the presence of Pd/C in a suitable solvent such as ethanol or tetrahydrofuran at room temperature followed by treatment with a compound of formula (IV) in a suitable solvent such as ethanol at elevated temperature.

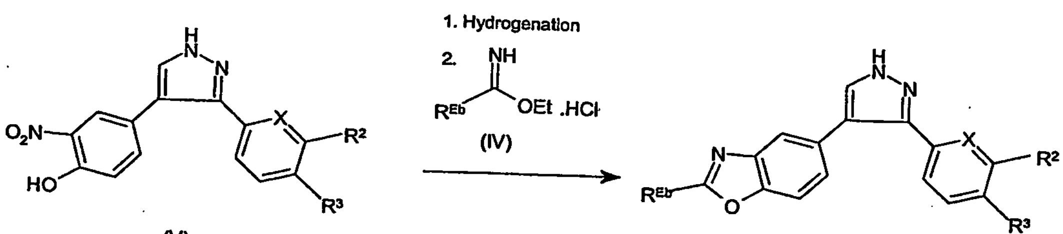
Scheme 2



(III) (Ib)

Compounds of formula (Ic), i.e. compounds of general formula (I) where the benzofused ring system including E is benzoxazol-5-yl, may be prepared from compounds of formula (V) according to reaction scheme 3 by treating (V) with hydrogen in the presence of Pd/C in a solvent such as ethanol or tetrahydrofuran at room temperature followed by treatment with a compound of formula (IV) in a suitable solvent such as ethanol at elevated temperature.

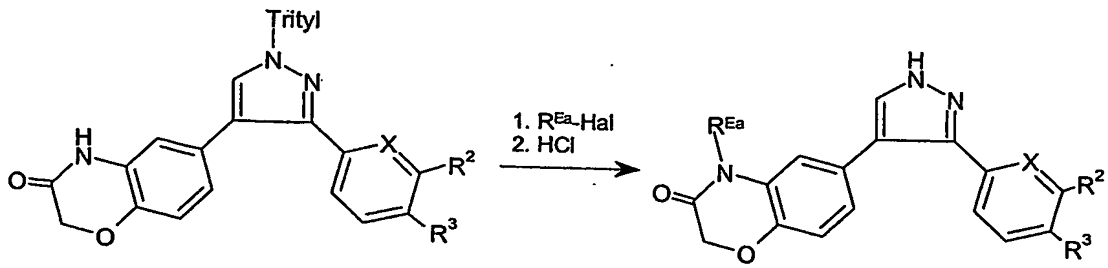
Scheme 3



(V) (Ic)

Compounds of formula (Id), i.e. compounds of general formula (I) where the benzofused ring system including E is 4H-benzo[1,4]oxazin-3-one-6-yl, may be prepared from compounds of formula (VI) according to reaction scheme 4 by treating (VI) with R^{Ea}-Hal (where Hal is halogen) in a solvent such as acetone in the presence of a base such as cesium carbonate at elevated temperature, followed by treatment with hydrochloric acid in a solvent such an alcohol at reflux.

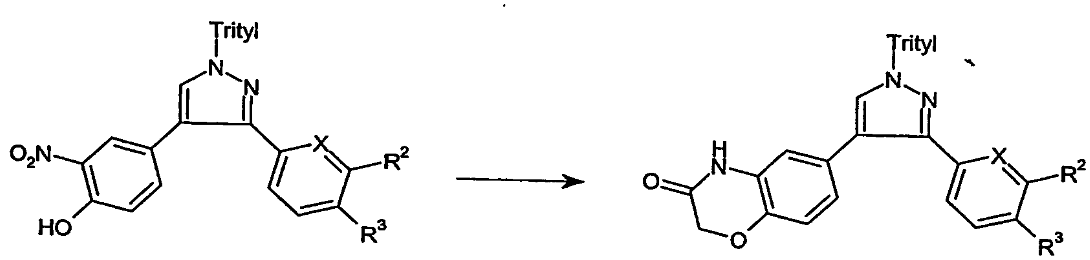
Scheme 4



(VI) (Id)

Compounds of formula (VI) may be prepared from compounds of formula (VII) according to reaction scheme 5 by treating (VII) with ethyl bromoacetate in a solvent such as acetone in the presence of a base such as cesium carbonate at room temperature followed by treatment with iron in acetic acid at elevated temperature.

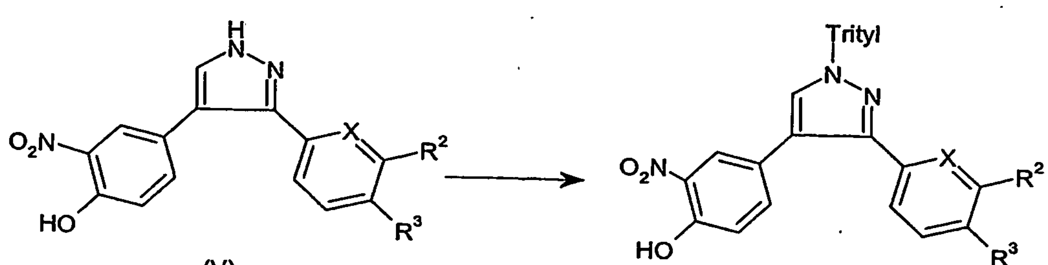
Scheme 5



(VII) (VI)

Compounds of formula (VII) may be prepared from compounds of formula (V) according to reaction scheme 6 by treating (V) with trityl chloride in a solvent such as methylene chloride in the presence of a base such as triethylamine at room temperature.

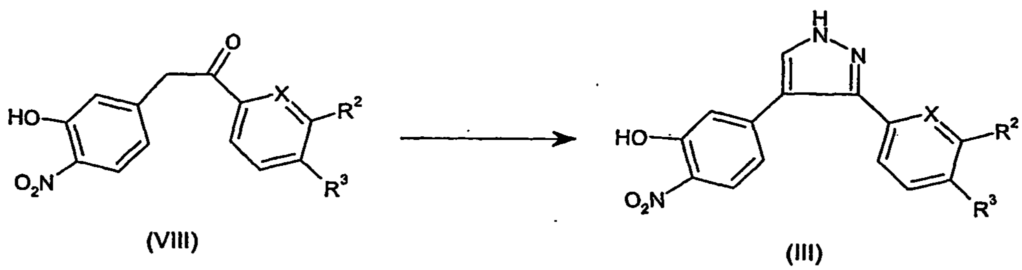
Scheme 6



(V) (VII)

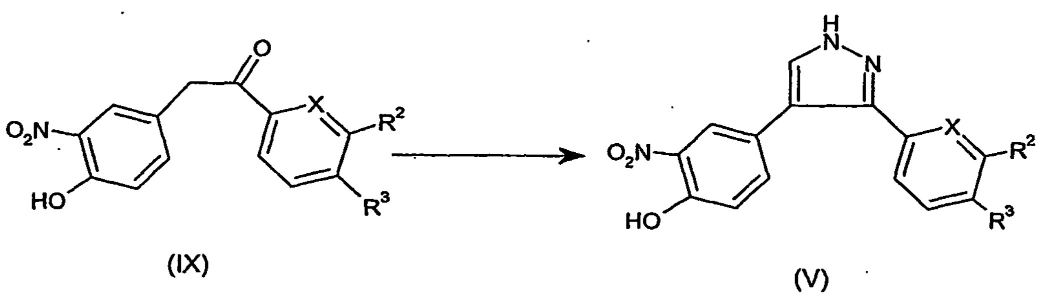
Compounds of formula (III) (see scheme 2) may be prepared from compounds of formula (VIII) according to reaction scheme 7 using analogous methods described for reaction scheme 1.

Scheme 7



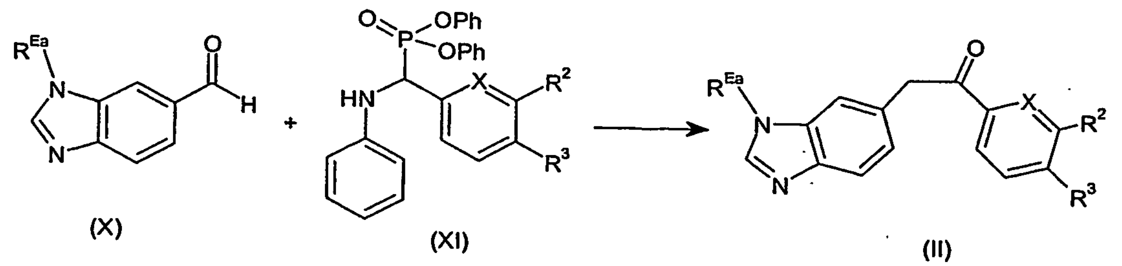
Compounds of formula (V) (see scheme 3) may be prepared from compounds of formula (IX) according to reaction scheme 8 using analogous methods described for reaction scheme 1.

Scheme 8



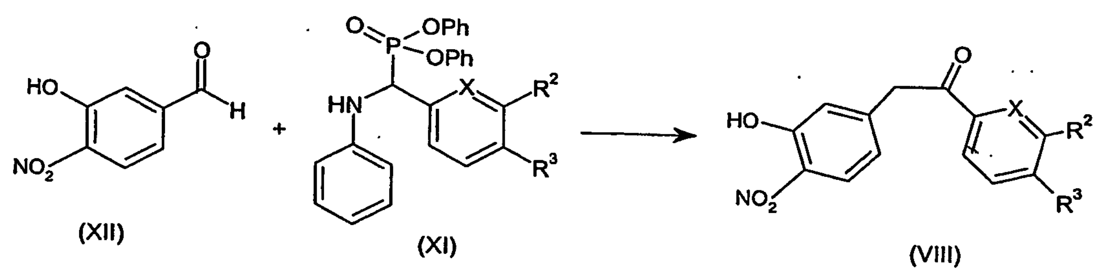
Compounds of formula (II) (see scheme 1) may be prepared according to reaction scheme 9 by reacting aldehydes of formula (X) with N,P acetals of formula (XI) followed by hydrolysis of the resulting enamine (see M. Journet, Tetrahedron Letters, 1998, 39, 1717-1720 and I. W. Davies *et al.*, J. Org. Chem., 2000, 65, 8415-8420). Preferred reaction conditions comprise treatment with a suitable base, such as caesium carbonate or potassium tert-butoxide, in a suitable solvent such as tetrahydrofuran and isopropyl alcohol. The enamine may be hydrolysed with hydrochloric acid.

Scheme 9



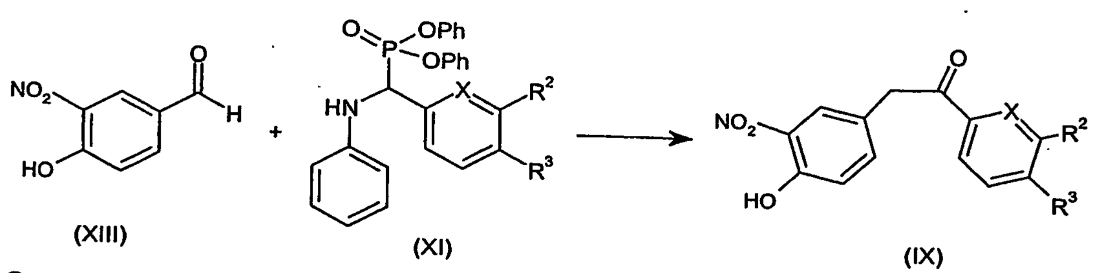
Compounds of formula (VIII) (see scheme 7) may be prepared from aldehydes of formula (XII) and N,P acetals of formula (XI) according to reaction scheme 10 using the same conditions as described for reaction Scheme 9.

Scheme 10



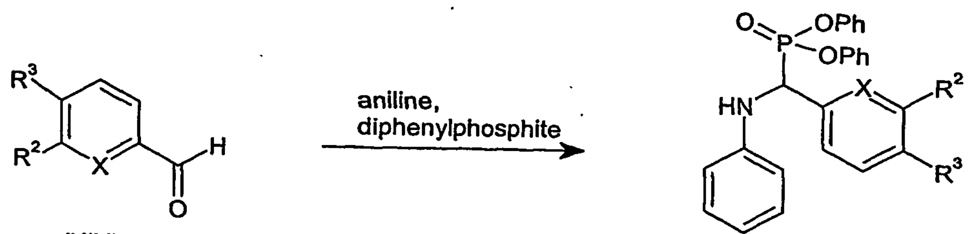
Compounds of formula (IX) (see scheme 8) may be prepared from 4-hydroxy-3-nitrobenzaldehyde (XIII) and N,P acetals of formula (XI) according to reaction scheme 11 using analogous methodology to that described for reaction Scheme10.

Scheme 11



Compounds of formula (XI) may be prepared from compounds of formula (XIV) according to reaction scheme 12 by treating compounds of formula (XIV) with aniline and diphenylphosphite in a suitable solvent such as isopropanol.

Scheme 12

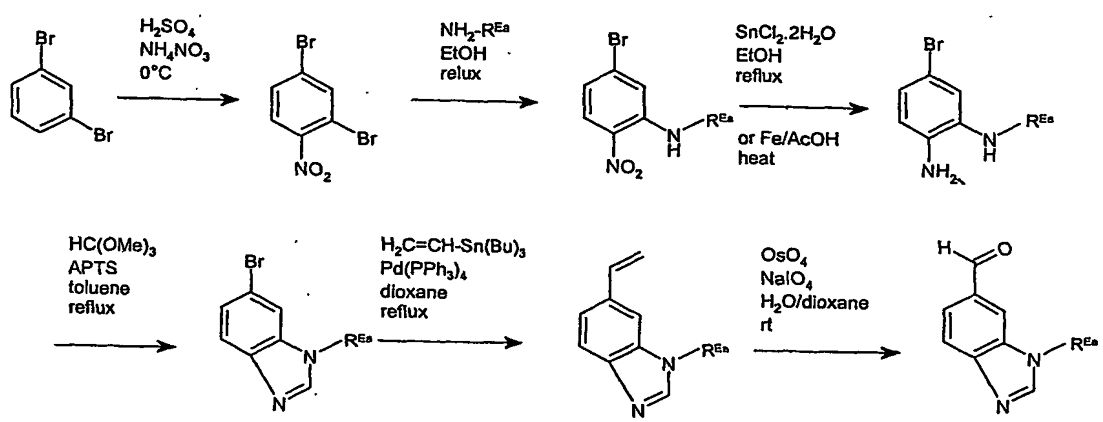


(XIV)

(XI)

Compounds of formula (X) (see scheme 9) may be prepared following the general methodology described in Scheme 13.

Scheme 13



(X)

Further details for the preparation of compounds of formula (I) are found in the examples.

The compounds of the invention may be prepared singly or as compound libraries comprising at least 2, for example 5 to 1,000 compounds, and more preferably 10 to 100 compounds. Libraries of compounds of the invention may be prepared by a combinatorial 'split and mix' approach or by multiple parallel synthesis using either solution phase or solid phase chemistry, by procedures known to those skilled in the art. Thus according to a further aspect there is provided a compound library comprising at least 2 compounds of the invention.

It will be appreciated that references herein to treatment extend to prophylaxis as well as the treatment of established conditions. It will further be appreciated that

references herein to treatment or prophylaxis of disorders characterised by the overexpression of TGF-β, shall include the treatment or prophylaxis of TGF-β associated disease such as fibrosis, especially liver and kidney fibrosis, cancer development, abnormal bone function and inflammatory disorders, and scarring.

Other pathological conditions which may be treated in accordance with the invention have been discussed in the introduction hereinbefore. The compounds of the present invention are particularly suited to the treatment of fibrosis and related conditions.

Compounds of the present invention may be administered in combination with other therapeutic agents, for example antiviral agents for liver diseases, or in combination with ACE inhibitors or angiotensin II receptor antagonists for kidney diseases.

The compounds of the invention may be administered in conventional dosage forms prepared by combining a compound of the invention with standard pharmaceutical carriers or diluents according to conventional procedures well known in the art. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation.

The pharmaceutical compositions of the invention may be formulated for administration by any route, and include those in a form adapted for oral, topical or parenteral administration to mammals including humans.

The compositions may be formulated for administration by any route. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, creams or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

The topical formulations of the present invention may be presented as, for instance, ointments, creams or lotions, eye ointments and eye or ear drops, impregnated dressings and aerosols, and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams.

The formulations may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and, if desired, conventional flavouring or colouring agents.

Suppositories will contain conventional suppository bases, e.g. cocoa-butter or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing.

Advantageously, agents such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry

lyophilized powder is then sealed in the vial and an accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The compound can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

The compositions may contain from 0.1% by weight, preferably from 10-60% by weight, of the active material, depending on the method of administration. Where the compositions comprise dosage units, each unit will preferably contain from 50-500 mg of the active ingredient. The dosage as employed for adult human treatment will preferably range from 100 to 3000 mg per day, for instance 1500 mg per day depending on the route and frequency of administration. Such a dosage corresponds to 1.5 to 50 mg/kg per day. Suitably the dosage is from 5 to 20 mg/kg per day.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular mammal being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound of the invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

No toxicological effects are indicated when a compound of the invention is administered in the above-mentioned dosage range.

All publications, including, but not limited to, patents and patent applications cited in this specification, are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

It will be appreciated that the invention includes the following further aspects. The preferred embodiments described for the first aspect extend these further aspects:

i) a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier or diluent;

ii) a compound of the invention for use as a medicament;

iii) a method of treatment or prophylaxis of a disorder selected from chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers (including diabetic ulcers, chronic ulcers, gastric ulcers, and duodenal ulcers), ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, atherosclerosis, peritoneal and sub-dermal adhesion, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, for example, hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol-induced hepatitis, haemochromatosis and primary biliary cirrhosis, restenosis, retroperitoneal fibrosis, mesenteric fibrosis, endometriosis, keloids, cancer, abnormal bone function, inflammatory disorders, scarring and photoaging, in mammals, which comprises administration to the mammal in need of such treatment, an effective amount of a compound of the invention; and

iv) a combination of a compound of the invention with an ACE inhibitor or an angiotensin II receptor antagonist.

The following non-limiting examples illustrate the present invention.

Abbreviations

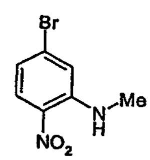
APTS	-	p-toluene sulfonic acid
Binap	-	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
CH_2Cl_2	-	dichloromethane
$CDCl_3$	-	deuterium chloroform
CH_3CN	-	acetonitrile
DMF.DMA	-	dimethylformamide dimethylacetal
EtOH	-	ethanol
EtOAc	-	ethyl acetate

iPrOH	-	isopropanol
MeOH	-	methanol
NaOH	-	sodium hydroxide
$NaHCO_3$	-	sodium bicarbonate
Na_2SO_4	-	sodium sulfate
THF	-	tetrahydrofuran
TEA	-	triethylamine
DME	-	dimethoxyethane
Pd/C	-	palladium on activated carbon
$Pd_2(dba)_3$	-	tris (dibenzylidene acetone)dipalladium
$SnCl_2.2H_2O$	-	tin(II) chloride dihydrate

Intermediate 1: 2,4-dibromo-nitrobenzene

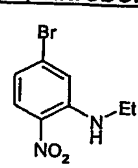
To an iced cold solution of 1,3-dibromobenzene (10g, 42.3mmol) in sulfuric acid (200ml) was added portionwise ammonium nitrate (3.39g, 42.3mmol) and the mixture was stirred at 0°C for 10 minutes and then poured into water. After extraction with CH_2Cl_2, the organic phase was washed with a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. After trituration with pentane, the title compound was obtained as a pale yellow solid (8g, 67.2%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 7.95 (s, 1H), 7.75 (d, 1H), 7.6 (d, 1H).

Intermediate 2: 4-bromo-2-(methylamino)-nitrobenzene



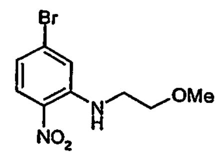
To a solution of intermediate 1 (8g, 28.5 mmol) in EtOH (200ml) was added a solution of methylamine 40% in water (200ml) and the mixture was heated under reflux for 2 hours and then cooled. The resulting precipitate was filtered and dried. The title compound was obtained as an orange solid (5g, 76%); m.p. 130-132°C.

Intermediate 3: 4-bromo-2-(ethylamino)-nitrobenzene



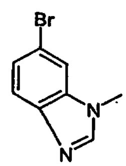
Intermediate 1 (6g, 28mmol) and ethylamine (solution 70% in water, 200ml) were reacted as described for intermediate 2 to afford the title compound as a yellow solid (5g, 99.9%); NMR H[1] (300MHz, $CDCl_3$, ppm) δ: 8.25 (ls, 1H), 8.1 (d, 1H), 7.05 (s, 1H), 6.85 (d, 1H), 3.25 (m, 2H), 1.3 (t, 3H).

Intermediate 4: 4-bromo-2-(2-methoxyethylamino)-nitrobenzene



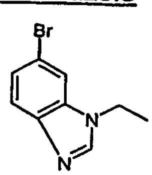
Intermediate 1 (6g, 28mmol) was reacted with 2-methoxyethylamine as described for intermediate 2, to afford the title compound as a solid (6g, 99.9%); NMR H[1] (300MHz, $CDCl_3$, ppm) δ: 8.3 (ls, 1H), 8.1 (d, 1H), 7.1 (s, 1H), 6.85 (d, 1H), 3.8 (m, 2H), 3.6 (m, 2H), 3.5 (s, 3H).

Intermediate 5: 1-methyl-6-bromo-benzimidazole



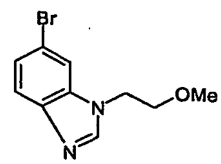
To a solution of intermediate 2 (5g, 21.6mmol) in EtOH (200ml) was added portionwise $SnCl_2.2H_2O$ (9.8g, 43mmol) and the mixture was heated under reflux for 4 hours and then concentrated under reduced pressure . The residue was treated with water (200ml) and 1N NaOH (100ml). After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure.
The residue was dissolved in toluene (50ml) and trimethylorthoformate (2.6ml, 24 mmol) and APTS (0.2g) were added and the mixture was heated under reflux for 2 hours and then concentrated under reduced pressure. The residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (95/5). The title compound was obtained as a cream powder (2.5g, 54.74%); m.p. 126-128°C.

Intermediate 6: 1-ethyl-6-bromo-benzimidazole



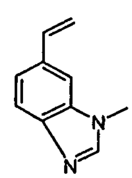
Intermediate 3 (5g, 22 mmol) was reacted as described for intermediate 5, to afford the title compound as a brown oil (2.3g, 47.23%); NMR H[1] (300MHz, $CDCl_3$, ppm) δ: 8.00 (s, 1H), 7.75 (d, 1H), 7.65 (s, 1H), 7.45 (d, 1H), 4.25 (q, 2H), 1.6 (t, 3H).

Intermediate 7: 1-(2-methoxyethyl)-6-bromo-benzimidazole



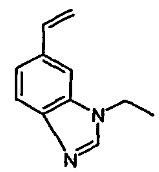
To a solution of intermediate 4 (6g, 22mmol) in acetic acid (100ml) at 60°C was added portionwise under vigorous stirring, iron (12g, 220mmol) and the mixture was heated at 60°C for 2 hours and then cooled. The reaction mixture was basified by addition of a solution of sodium hydroxide, filtered on a celite pad and the filtrate was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in toluene (80ml) and trimethylorthoformate (3.5ml, 32mmol) and APTS (0.6g) were added and the mixture was heated under reflux overnight and then concentrated under reduced pressure. The residue was purified by chromatography on silicagel eluting with CH_2Cl_2/MeOH (9/1). The title compound was obtained as an oil (6g, 96.07%); NMR H[1] (300MHz, $CDCl_3$, ppm) δ: 8.1 (s, 1H), 7.8 (d, 1H), 7.7 (s, 1H), 7.5 (d, 1H), 4.4 (t, 2H), 3.85 (t, 2H), 3.4 (s, 3H).

Intermediate 8: 1-methyl-6-vinyl-benzimidazole



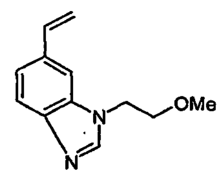
To a solution of intermediate 5 (2.5g, 11.8 mmol) in dioxane (100ml) were added tributyl(vinyl)tin (5.2ml, 18 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.69g, 0.5mmol) and the mixture was heated under reflux for 24 hours and then concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (95/5). The title compound was obtained as an oil (1.8g, 96.1%); NMR H[1] (300MHz, $CDCl_3$, ppm) δ: 7.9 (s, 1H), 7.75 (d, 1H), 7.4 (m, 2H), 6.85 (dd, 1H), 5.8 (d, 1H), 5.25 (d, 1H), 3.85 (s, 3H).

Intermediate 9: 1-ethyl-6-vinyl-benzimidazole



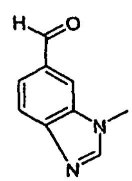
Intermediate 6 (2.3g, 10mmol) was reacted as described for intermediate 8 to afford the title compound as an oil (1.5g, 85.31%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 7.9 (s, 1H), 7.75 (d, 1H), 7.4 (m, 2H), 6.95 (m, 1H), 5.8 (m, 1H), 5.25 (m, 1H), 4.2 (q, 2H), 1.55 (t, 3H).

Intermediate 10: 1-(2-methoxyethyl)-6-vinyl-benzimidazole



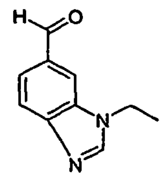
Intermediate 7 (6g, 23.5mmol) was reacted as described for intermediate 8 to afford the title compound as an oil (3.5g, 73.64%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 7.95 (s, 1H), 7.75 (d, 1H), 7.4 (m, 2H), 6.9 (m, 1H), 5.85 (d, 1H), 5.25 (m, 1H), 4.35 (t, 2H), 3.75 (t, 2H), 3.3 (s, 3H).

Intermediate 11: 1-methyl-6-formyl-benzimidazole



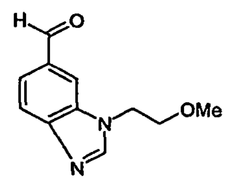
To a solution of intermediate 8 (1.8g, 11.4mmol) in dioxane (100ml) and water (14ml) was added osmium tetroxide (solution 2.5% in water, 6ml) and the mixture was stirred for 5 minutes at room temperature. Then sodium periodate (5.1g, 23.9mmol) was added and the mixture was stirred at room temperature for 3 hours and then poured into water. The aqueous phase was extracted with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The title compound was obtained as a cream powder (1g, 55%); m.p. 132-134°C.

Intermediate 12: 1-ethyl-6-formyl-benzimidazole



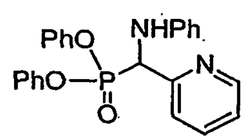
Intermediate 9 (1.5g, 8.8mmol) was reacted as described for intermediate 11 to afford the title compound as an oil (1.3g, 85.67%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 10.1 (s, 1H), 8.15 (s, 1H), 8.05 (s, 1H), 7.95 (d, 1H), 7.85 d, 1H), 4.3 (q, 2H), 1.6 (t, 3H).

Intermediate 13: 1-(2-methoxyethyl)-6-formyl-benzimidazole



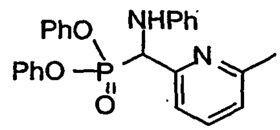
Intermediate 10 (3.5g,17.33mmol) was reacted as described for intermediate 11 to afford the title compound as an oil (1.3g, 36.78%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 10.05 (s, 1H), 8.15 (s, 1H), 8 (s, 1H), 7.9 (d, 1H), 7.8 (d, 1H), 4.4 (t, 2H), 3.7 (t, 2H), 3.3 (s, 3H).

Intermediate 14: (phenylamino -pyridin-2-yl-methyl)-phosphonic acid diphenylester



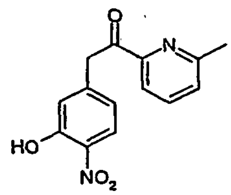
To a solution of 2-pyridinecarboxaldehyde (15g, 140mmol) in iPrOH (200ml) were added aniline (1.2eq, 15.65g, 168mmol) and diphenylphosphite (37.5ml, 197mmol). The reaction mixture crystallised after 30min at room temperature. Isopropanol (300 ml) was added and the mixture was allowed to stir for 2h. The resulting solid was filtered and dried to give the title compound as a white solid (56g , 96.15%); m.p. 130-132°C.

Intermediate 15: [(6-Methylpyridin-2-yl)-phenylamino-methyl]-phosphonic acid diphenylester



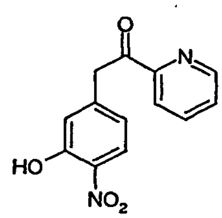
6-Methyl-2-pyridinecarboxaldehyde (10g, 83mmol) was reacted with aniline and diphenylphosphite as described for Intermediate 14, to afford the title compound as a white solid (40g, 99.53%); m.p. 110-112°C.

Intermediate 16: 2-[3-hydroxy-4-nitrophenyl]-1-[6-methylpyridin-2-yl]-ethanone



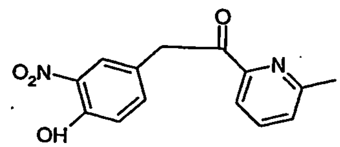
To a solution of 3-hydroxy-4-nitro-benzaldehyde (15g, 90mmol) and intermediate 15 (38.62g, 90mmol) in THF (200ml) and iPrOH (200ml) was added cesium carbonate (88g, 27mmol) and the mixture was stirred at room temperature overnight. The mixture was acidified to pH3 by addition of a solution of 4N HCl, and allowed to stir at room temperature for 2 hours and then poured into water. After neutralisation with a solution of 1N NaOH, the aqueous phase was extracted with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (8/2). The title compound was obtained as an orange solid (15g, 61.4%); m.p. 128-130°C.

Intermediate 17: 2-[3-hydroxy-4-nitrophenyl]-1-[pyridin-2-yl]-ethanone



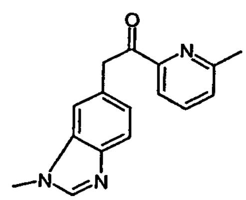
4-Nitro-3-(t-butyl-dimethylsilyloxy)-benzaldehyde (5g, 17.8mmol) and intermediate 14 (7.4g, 17.8mmol) were reacted as described for intermediate 16 to afford, after chromatography on silica gel (CH_2Cl_2/MeOH, 98/2), the title compound as a brown solid (3g, 64.85%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 10.55 (s, 1H), 8.7 (d, 1H), 8.05 (m, 2H), 7.85 (t, 1H), 7.5 (m, 1H), 7.1 (s, 1H), 6.95 (d, 1H), 4.55 (s, 2H).

Intermediate 18: 2-[4-hydroxy-3-nitrophenyl]-1-[6-methylpyridin-2-yl]-ethanone



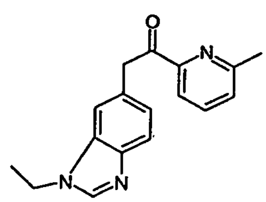
Intermediate 15 (14.2g, 33mmol) and 4-hydroxy-3-nitro-benzaldehyde (5g, 30 mmol) were reacted as described for intermediate 16 to afford, after chromatography on silica gel (CH_2Cl_2), the title compound as a brown oil (3.5g, 42.97%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 10.25 (s, 1H), 8.7 (d, 1H), 8.2 (s, 1H), 7.7 (m, 1H), 7.15 (m, 1H), 6.9 (t, 1H), 6.8 (d, 1H), 4.5 (s, 2H), 2.65 (s, 3H).

Intermediate 19: 2-[1-methyl-benzimidazol-6-yl]-1-[6-methylpyridin-2-yl]-ethanone



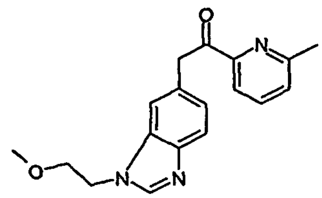
Intermediate 11 (3g, 18.8 mmol) and intermediate 15 (9.68g, 22.5mmol) were reacted as described for intermediate 16 to afford the title compound as a solid (1.1g, 22.14%); m.p. 96-98°C.

Intermediate 20: 2-[1-ethyl-benzimidazol-6-yl]-1-[6-methylpyridin-2-yl]-ethanone



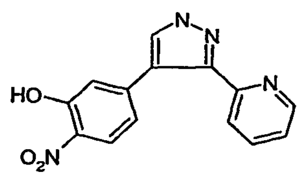
Intermediate 12 (1.3g, 7.5mmol) and intermediate 15 (3.86g, 9mmol) were reacted as described for intermediate 16 to afford the title compound as a brown oil (1g, 47.97%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 7.9 (s, 1H), 8 (m, 1H), 7.85 (m, 1H), 7.55 (s, 1H), 7.35 (m, 1H), 7.2 (m, 1H), 6.9 (m, 1H), 4.7 (s, 2H), 4.25 (q, 2H), 2.65 (s, 3H), 1.55 (t, 3H).

Intermediate 21: 2-[1-(2-methoxyethyl)-benzimidazol-6-yl]-1-[6-methylpyridin-2-yl]-ethanone



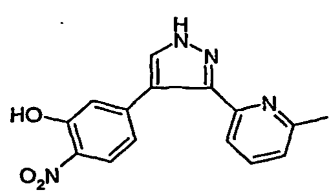
Intermediate 13 (1.3g, 6.4mmol) and intermediate 15 (3.29g, 7.6mmol) were reacted as described for intermediate 16 to afford the title compound as a brown oil (1.2g, 60.94%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 8.00 (s, 1H), 7.9 (m, 1H), 7.75 (m, 2H), 7.45 (s, 1H), 7.3 (m, 1H), 7.1 (m, 1H), 4.7 (s, 2H), 4.3 (t, 2H), 3.7 (t, 2H), 3.3 (s, 3H), 2.6 (s, 3H).

Intermediate 22: 4-[3-hydroxy-4nitro-phenyl]-3-[pyridin-2-yl]-1H-pyrazole



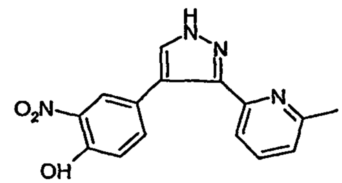
To a solution of intermediate 17 (2g, 7.7mmol) in THF (80ml) and acetic acid (1ml) was added DMF.DMA (1.5ml) and the mixture was stirred at room temperature for 5 hours. Then hydrazine hydrate (3ml) was added and the mixture was stirred at room temperature for 5 hours and then poured into water. The aqueous phase was extracted with CH_2Cl_2, the organic phase dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (95/5).The title compound was obtained as a brown solid (1g, 46.1%); m.p. 170-172°C; [APCI MS] m/z 283 MH^+.

Intermediate 23: 4-[3-hydroxy-4nitro-phenyl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



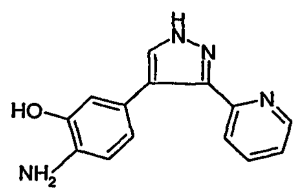
Intermediate 16 (1g, 3.67mmol) was reacted as described for intermediate 22, to afford, after chromatography on silicagel (CH_2Cl_2/MeOH, 9/1), the title compound as a solid (1.1g, 99.9%); [APCI MS] m/z= 297 MH^+.

Intermediate 24: 4-[4-hydroxy-3-nitro-phenyl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



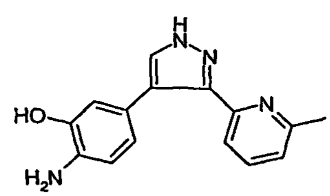
Intermediate 18 (3.5g, 12.87mmol) was reacted as described for intermediate 22, to afford, after chromatography on silica gel (CH_2Cl_2/MeOH, 9/1), the title compound as a yellow oil (1.2g, 31.5%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 10.35 (s, 1H), 8.2 (m, 1H), 7.7 (m, 1H), 7.65 (s, 1H), 7.4 (m, 2H), 7.2 (m, 1H), 7.1 (m, 1H), 2.6 (s, 3H).

Intermediate 25: 4-[4-amino-3-hydroxy-phenyl]-3-[pyridin-2-yl]-1H-pyrazole



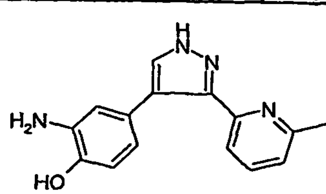
To a solution of intermediate 22 (1g, 3.5mmol) in EtOH (100ml) and THF (50ml) was added Pd/C 10% (100mg), and the mixture was hydrogenated at room temperature under 1.5 bars for 5 hours. The reaction mixture was purged with argon, the catalyst was filtered off and the filtrate was concentrated under reduced pressure. The title compound was obtained as a red solid (0.68g, 76.1%); [APCI MS] m/z : 253 MH^+.

Intermediate 26: 4-[4-amino-3-hydroxy-phenyl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



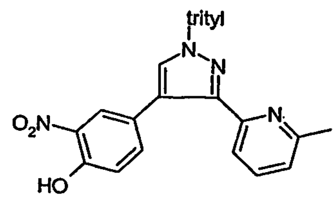
Intermediate 23 (1.1g, 3.7mmol) was reacted as described for intermediate 25 to afford, the title compound as an orange oil (0.9g, 91.04%); [APCI MS] m/z= 267 MH^+.

Intermediate 27: 4-[3-amino-4-hydroxy-phenyl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



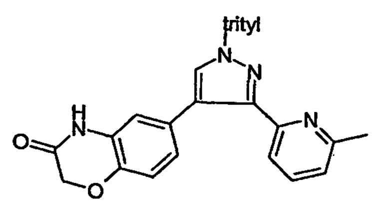
Intermediate 24 (1.2g, 4.05mmol) was reacted as described for intermediate 25 to afford, after chromatography on silica gel (CH_2Cl_2/MeOH, 9/1),the title compound as a yellow oil (0.5g, 46.36%); [APCI MS] m/z= 267 MH^+.

Intermediate 28: 4-[4-hydroxy-3-nitro-phenyl]-3-[6-methylpyridin-2-yl]-1-trityl-1H-pyrazole



To a solution of intermediate 24 (4.47g, 16 mmol) in CH_2Cl_2 (100 ml) were added triethylamine (3.4 ml, 24 mmol) and trityl chloride (6.8g, 24 mmol) and the mixture was heated under reflux for 16 hours and then poured into water. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The title compound was obtained as an oil (7.5g, 84.24%); NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 8.1 (s, 1H), 7.45 (m, 3H), 7.35 to 7.15 (m, 15H), 6.95 (d, 1H), 6.8 (d, 1H), 2.35 (s, 3H).

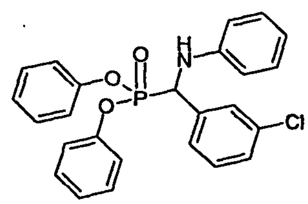
Intermediate 29 :4-[4H-benzo[1,4]oxazin-3-one-6-yl]-3-[6-methylpyridin-2-yl]-1-trityl-1H-pyrazole



To a solution of intermediate 28 (7.5g, 13.6 mmol) in acetone (200ml) were added cesium carbonate (7.3g, 24 mmol) and ethyl bromo acetate (4g, 24 mmol) and the mixture was stirred at room temperature for 2 hours and then poured into water. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (CH_2Cl_2). The resulting yellow oil (5g, 8 mmol) was dissolved in acetic acid (250 ml) and iron (4.5g, 80 mmol) was added portionwise. The mixture was heated at 60°C for 3 hours and then poured into water. The mixture was basified with aqueous NaOH and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered on a celite pad, and concentrated under reduced pressure. The title

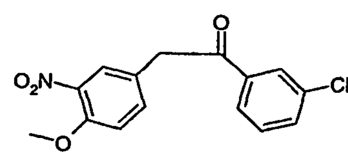
compound was obtained as a brown oil (3.2g, 42.98%); [APCI MS] m/z 549 MH^+ and 307 MH^+ (-trityl).

Intermediate 30: [(3-chlorophenyl)(phenylamino)methyl] phosphonic acid diphenylester



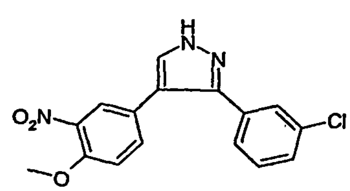
3-Chloro-benzaldehyde (26.57g, 189mmol) was reacted with aniline and diphenylphosphite as described for Intermediate 14, to afford the title compound as a white solid (82.48g, 99.7%); m.p. 130°C.

Intermediate 31: 1-(3-chlorophenyl)-2-[4-(methoxy)-3-nitrophenyl]ethanone



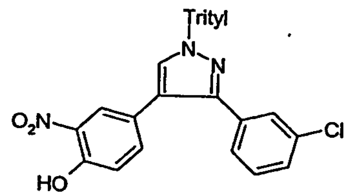
Intermediate 30 (29g, 66.24mmol) and 4-methoxy-3-nitro-benzaldehyde (10g, 55.2mmol) were reacted as described for intermediate 16 to afford, after chromatography on silica gel (cyclohexane/AcOEt 9/1), the title compound as a beige solid (16.8g, 99.6%); [APCI MS] m/z= 304 MH^-.

Intermediate 32 : 3-(3-chlorophenyl)-4-[4-(methoxy)-3-nitrophenyl]-1*H*-pyrazole



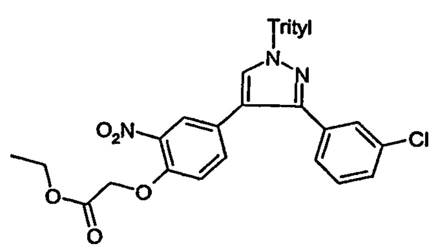
Intermediate 31 (10g, 32.71mmol) was reacted as described for intermediate 22 to afford, after chromatography on silica gel (cyclohexane/AcOEt 9/1),the title compound as a yellow solid (10.2g, 94.6%); [APCI MS] m/z= 328 MH^-.

Intermediate 33 : 3-(3-chlorophenyl)-4-[4-hydroxy-3-nitrophenyl]-1-trityl-1*H*-pyrazole



A mixture of intermediate 32 (10g, 30.3mmol) and lithium chloride (12.85g, 303mmol) in DMF (120mL) was heated under reflux for 16 hours. The reaction mixture was quenched with ammonium chloride and extracted with CH_2Cl_2 . The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. After chromatography on silica gel (cyclohexane/AcOEt 9/1), 3-(3-chlorophenyl)-4-[4-(hydroxy)-3-nitrophenyl]-1*H*-pyrazole was obtained as a yellow solid (9.5g). This compound was reacted in the next step without purification. To a solution of 3-(3-chlorophenyl)-4-[4-(hydroxy)-3-nitrophenyl]-1*H*-pyrazole (9.5g, 30.3mmol) in CH_2Cl_2 (100 mL) were added triethylamine (4.6ml, 45.45 mmol) and trityl chloride (12.67g, 45.45mmol) and the mixture was stirred at room temperature for 18 hours and then poured into water. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The title compound was obtained, after purification by chromatography on silica gel (cyclohexane /AcOEt 9/1) as a yellow solid (5.4g, 32%). NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 10.48 (brs, 1H), 7.92 (d, J=2.26Hz, 1H), 7.41 (dd, J=1.70, 1.70Hz, 1H), 7.40-7.13 (m, 20H), 7.00 (d, J=8.67Hz , 1H).

Intermediate 34 : ethyl ({4-[3-(3-chlorophenyl)-1-trityl-1*H*-pyrazol-4-yl]-2-nitrophenyl}oxy)acetate

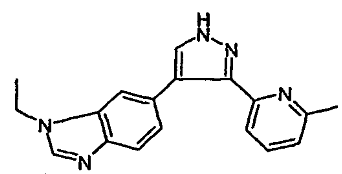


To a solution of intermediate 33 (5.8g, 10.39mmol) in acetone (130ml) were added cesium carbonate (4.67g, 14.33mmol) and ethyl bromoacetate (1.6mL, 14.33mmol) and the mixture was stirred at room temperature for 24 hours and then poured into water. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (cyclohexane /AcOEt 9/1) to afford the title compound as yellow solid

(6.6g, 98.6%). NMR H^1 (300MHz, $CDCl_3$, ppm) δ: 7.78 (d, J=2.26Hz, 1H), 7.51-7.48. (m, 1H), 7.44 (s, 1H), 7.40-7.17 (m, 20H), 6.90 (d, J=8.67Hz , 1H), 4.77 (s, 2H), 4.79 (q, J=7.16Hz, 2H), 1.30 (t, J=7.16Hz, 3H).

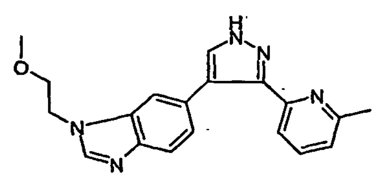
Examples

Example 1: 4-[1-ethyl-benzimidazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



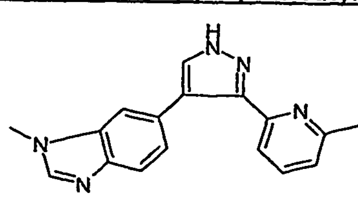
To a solution of intermediate 20 (0.5g, 1.8mmol) in THF (80ml) and acetic acid (0.3ml) was added DMF.DMA (0.4ml) and the mixture was stirred at room temperature for 4 hours. Then hydrazine hydrate (0.7ml) was added and the mixture was stirred at room temperature for 48 hours and then poured into water. The aqueous phase was extracted with CH_2Cl_2, the organic phase dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (9/1). After crystallisation from ethyl acetate, the title compound was obtained as cream crystals (0.17g, 31.31%); m.p. 182-184°C; [APCI MS] m/z= 304 MH^+.

Example 2: 4-[1-(2-methoxyethyl)-benzimidazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



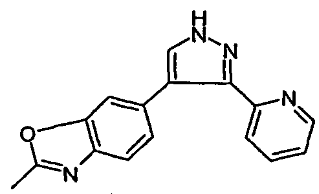
Intermediate 21 (0.6g, 1.9 mmol) was reacted as described for example 1 to afford , after crystallisation from ethanol, the title compound as crystals (0.3g, 47.41%); m.p. 172-174°C; [APCI MS] m/z= 334 MH^+.

Example 3: 4-[1-(methyl)-benzimidazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



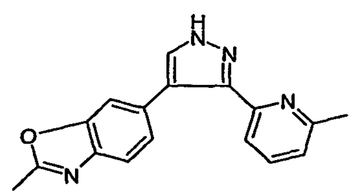
Intermediate 19 (0.5g, 1.9 mmol) was reacted as described for example 1 to afford, after trituration with pentane, the title compound a solid (0.21g, 38.51%); [APCI MS] m/z= 290 MH^{+}.

Example 4: 4-[2-(methyl)-benzoxazol-6-yl]-3-[pyridin-2-yl]-1H-pyrazole



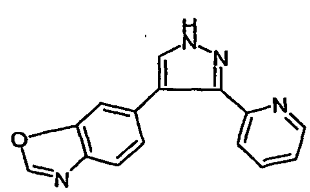
To a solution of intermediate 25 (0.504g, 2mmol) in EtOH (20ml) was added ethyl acetimidate hydrochloride (0.247g, 2 mmol) and the mixture was heated under reflux for 4 hours and then concentrated. The residue was treated with water and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (9/1). After trituration with pentane, the title compound was obtained as a cream solid (0.42g, 76.1%); m.p. 138-140°C; [APCI MS] m/z= 277 MH^{+}.

Example 5: 4-[2-(methyl)-benzoxazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



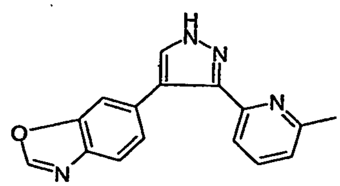
Intermediate 26 (0.45g, 1.69mmol) and ethyl acetimidate hydrochloride (0.315g, 2.5mmol) were reacted as described for example 4 to afford, after chromatography on silicagel, eluting with CH_2Cl_2/MeOH (9/1), the title compound as a cream oil (0.3g, 61.15%); [APCI MS] m/z= 291 MH^{+}.

Example 6: 4-[benzoxazol-6-yl]-3-[pyridin-2-yl]-1H-pyrazole



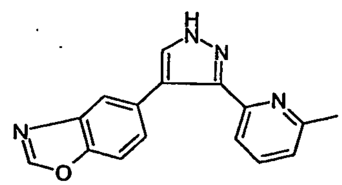
Intermediate 25 (0.504g, 2mmol) and ethyl formimidate hydrochloride (0.219g, 2 mmol) were reacted as described for example 4 to afford, after trituration with pentane, the title compound as a cream solid (0.27g, 51.53%); m.p. 176-178°C; [APCI MS] m/z= 263 MH^+.

Example 7: 4-[benzoxazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



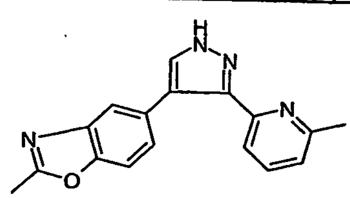
Intermediate 26 (0.45g, 1.69 mmol) and ethyl formimidate hydrochloride (0.278g, 2.5 mmol) were reacted as described for example 4, to afford, after chromatography on silicagel, eluting with CH_2Cl_2/MeOH (9/1), the title compound as a cream oil (0.14g, 29.98%); [APCI MS] m/z= 277 MH^+.

Example 8: 4-[benzoxazol-5-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



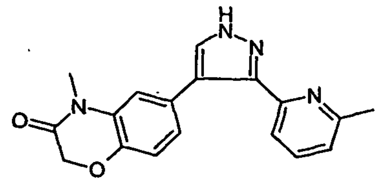
Intermediate 27 (0.25g, 0.94mmol) and ethyl formimidate hydrochloride (0.155g, 1.41mmol) were reacted as described for example 4 to afford, after crystallisation from CH_3CN/CH_2Cl_2 , the title compound as yellow crystals (0.1g, 38.55%); m.p. 236-238°C; [APCI MS] m/z= 277 MH^+.

Example 9: 4-[2-methyl-benzoxazol-5-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole



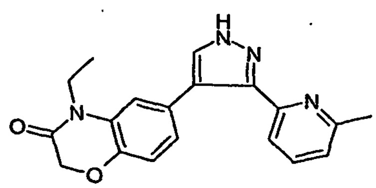
Intermediate 27 (0.25g, 0.94mmol) and ethyl acetimidate hydrochloride (0.154g, 1.41 mmol) were reacted as described for example 4 to afford, after crystallisation from CH_3CN/CH_2Cl_2, the title compound as yellow crystals (0.04g, 14.67%); m.p. 208-210°C; [APCI MS] m/z= 291 MH^+.

Example 10: 4-[4-methyl-4H-benzo[1,4]oxazin-3-one-6-yl]-3-(6-methylpyridin-2-yl)-1H-pyrazole



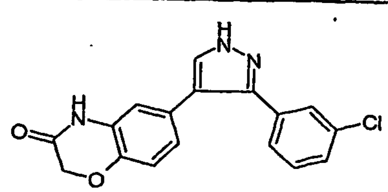
To a solution of intermediate 29 (1g, 1.8mmol) in acetone (60ml) were added cesium carbonate (0.88g, 2.7 mmol) and methyl iodide (0.17ml, 2.7 mmol) and the mixture was heated at 60°C for 4 hours and then poured into water. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in a mixture of methanol (50ml) and 1N HCl (15ml) and the mixture was heated under reflux for 3 hours. On cooling the mixture was poured into water, neutralised with a solution of 1N NaOH and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 95/5). After trituration with pentane, the title compound was obtained as a cream solid (0.1g, 17.12%); m.p. 204-206°C; [APCI MS] m/z 321 MH^+.

Example 11 : 4-[4-ethyl-4H-benzo[1,4]oxazin-3-one-6-yl]-3-(6-methylpyridin-2-yl)-1H-pyrazole



Intermediate 29 (1g, 1.8mmol) was reacted with ethyl iodide as described for example 10 to afford, after trituration with pentane, the title compound as a cream solid (0.16g, 26.25%); m.p. 182-184°C; [APCI MS] m/z 335 MH^+.

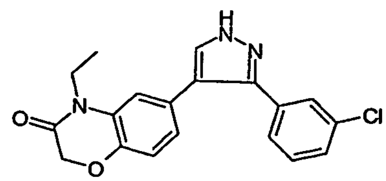
Example 12: 4-[4H-benzo[1,4]oxazin-3-one-6-yl]-3-(3-chlorophenyl)-1H-pyrazole



Intermediate 34 (6.5g, 10.09mmol) was dissolved in acetic acid (300 ml) at 60°C and iron (5.6g, 100.9mmol) was added portionwise. The mixture was stirred at 60°C for 3

hours and then poured into water. After extraction with ethyl acetate, the organic phase was dried over Na_2SO_4, filtered on a celite pad, and concentrated under reduced pressure. Purification by chromatography on silica gel afforded the title compound as a white solid (3g, 91.5%); [ES MS] m/z= 326 MH^+.

Example 13 : 4-[4-ethyl-4H-benzo[1,4]oxazin-3-one-6-yl]-3-(3-chlorophenyl)-1H-pyrazole



Example 13 (3g, 9.23mmol) was reacted with triethylamine (1.95ml, 13.85mmol) and trityl chloride (3.86g, 13.85mmol), the mixture was heated under reflux for 24 hours. The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to afford, after chromatography on silica gel (cyclohexane/AcOEt 9/1), the 4-[4H-benzo[1,4]oxazin-3-one-6-yl]-3-(3-chlorophenyl)-1-trityl-1H-pyrazole as a yellow solid. To a solution of 4-[4H-benzo[1,4]oxazin-3-one-6-yl]-3-(3-chlorophenyl)-1-trityl-1H-pyrazole (5.36g, 9.43mmol) in DMF(50ml) was added cesium carbonate (4.61g, 14.15mmol) and ethyl iodide (1.13ml, 14.15mmol). The mixture was stirred at room temperature overnight. The reaction mixture was poured in water, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (cyclohexane/AcOEt 6/4) to afford 4-[4-ethyl-4H-benzo[1,4]oxazin-3-one-6-yl]-3-(3-chlorophenyl)-1-trityl-1H-pyrazole as a white solid. This compound (4.2g) was reacted with a mixture of MeOH/ 1N HCl (3/2, 80ml), the reaction mixture was heated under reflux for 3 hours. On cooling the mixture was poured into water, neutralised with a solution of 1N NaOH and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 95/5) to give the title compound, after re-crystallisation from acetonitrile, as a white solid (1.6g, 48%); m.p. 162°C ;[ES MS] m/z= 354 MH^+.

Biology

The biological activity of the compounds of the invention may be assessed using the following assays:

Assay 1 (Cellular transcriptional assay)

The potential for compounds of the invention to inhibit TGF- β signalling may be demonstrated, for example, using the following *in vitro* assay.

The assay was performed in HepG2 cells stably transfected with the PAI-1 promoter (known to be a strong TGF-β responsive promoter) linked to a luciferase (firefly) reporter gene. The compounds were selected on their ability to inhibit luciferase activity in cells exposed to TGF-β. In addition, cells were transfected with a second luciferase (Renilla) gene which was not driven by a TGF-β responsive promoter and was used as a toxicity control.

96 well microplates were seeded, using a multidrop apparatus, with the stably transfected cell line at a concentration of 35000 cells per well in 200 μl of serum-containing medium. These plates were placed in a cell incubator.

18 to 24 hours later (Day 2), cell-incubation procedure was launched. Cells were incubated with TGF-β and a candidate compound at concentrations in the range 50 nM to 10 μM (final concentration of DMSO 1%). The final concentration of TGF-β (rhTGFβ-1) used in the test was 1 ng/mL. Cells were incubated with a candidate compound 15-30 mins prior to the addition of TGF-β. The final volume of the test reaction was 150 μl. Each well contained only one candidate compound and its effect on the PAI-1 promoter was monitored.

Columns 11 and 12 were employed as controls. Column 11 contained 8 wells in which the cells were incubated in the presence of TGF-β, without a candidate compound. Column 11 was used to determine the 'reference TGF-β induced firefly luciferase value' against which values measured in the test wells (to quantify inhibitory activity) were compared. In wells A12 to D12, cells were grown in medium without TGF-β. The firefly luciferase values obtained from these positions are representative of the 'basal firefly luciferase activity'. In wells E12 to H12, cells were incubated in the presence of TGF-β and 500 μM CPO (Cyclopentenone, Sigma), a cell toxic compound. The toxicity was revealed by decreased firefly and renilla luciferase activities (around 50 % of those obtained in column 11).

12 to 18 hours later (day 3), the luciferase quantification procedure was launched. The following reactions were performed using reagents obtained from a Dual Luciferase Assay Kit (Promega). Cells were washed and lysed with the addition of

10 µl of passive lysis buffer (Promega). Following agitation (15 to 30 mins), luciferase activities of the plates were read in a dual-injector luminometer (BMG lumistar). For this purpose, 50 µl of luciferase assay reagent and 50 µl of 'Stop & Glo' buffer were injected sequentially to quantify the activities of both luciferases. Data obtained from the measurements were processed and analysed using suitable software. The mean Luciferase activity value obtained in wells A11 to H11 (Column 11, TGF-βonly) was considered to represent 100% and values obtained in wells A12 to D12 (cells in medium alone) gave a basal level (0%). For each of the compounds tested, a concentration response curve was constructed from which an IC_{50} value was determined graphically.

<u>Assay 2 (Alk5 Fluorescence Polarization Assay)</u>

Kinase inhibitor compounds conjugated to fluorophores, can be used as fluorescent ligands to monitor ATP competitive binding of other compounds to a given kinase. The increase in depolarization of plane polarized light, caused by release of the bound ligand into solution, is measured as a polarization/anisotropy value. This protocol details the use of a rhodamine green-labelled ligand for assays using recombinant GST-ALK5 (residues 198-503).

Assay buffer components: 62.5 mM Hepes pH 7.5 (Sigma H-4034), 1 mM DTT (Sigma D-0632), 12.5 mM $MgCl_2$ (Sigma M-9272), 1.25 mM CHAPS (Sigma C-3023).

Protocol: Solid compound stocks were dissolved in 100% DMSO to a concentration of 1 mM and transferred into column 1, rows A-H of a 96-well, U bottom, polypropylene plate (Costar #3365) to make a compound plate. The compounds were serially diluted (3-fold in 100% DMSO) across the plate to column 11 to yield 11 concentrations for each test compound. Column 12 contained only DMSO. A Rapidplate™-96 was used to transfer 1 µl of sample from each well into a 96-well, black, U-bottom, non-treated plate (Costar #3792) to create an assay plate.

ALK5 was added to assay buffer containing the above components and 1 nM of the rhodamine green-labelled ligand so that the final ALK5 concentration was 10 nM based on active site titration of the enzyme. The enzyme/ligand reagent (39 µl) was added to each well of the previously prepared assay plates. A control compound (1 µl) was added to column 12, rows E-H for the low control values. The plates were

read immediately on a LJL Acquest fluorescence reader (Molecular Devices, serial number AQ1048) with excitation, emission, and dichroic filters of 485nm, 530 nm, and 505 nm, respectively. The fluorescence polarization for each well was calculated by the Acquest reader and then imported into curve fitting software for construction of concentration response curves. The normalized response was determined relative to the high controls (1 µl DMSO in column 12, rows A-D) and the low controls (1 µl of control compound in column 12, rows E-H). An IC_{50} value was then calculated for each compound

Using the above assays all Examples of the invention show ALK5 receptor modulator activity (having IC_{50} values in the range of 1 to 100nM) and TGF-β cellular activity (having IC_{50} values in the range of 0.001 to 10µM).

4-[1-Ethyl-benzimidazol-6-yl]-3-[6-methylpyridin-2-yl]-1H-pyrazole (Example 1) showed an ALK5 receptor modulator activity of 25 nM and TGF-β cellular activity of 20 nM.

4-[4-Methyl-4H-benzo[1,4]oxazin-3-one-6-yl]-3-(6-methylpyridin-2-yl)-1H-pyrazole (Example 10) showed an ALK5 receptor modulator activity of 32 nM and TGF-β cellular activity of 20 nM.